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**Bacterial Transformation Lab**

Ms. Ottolini, AP Biology

**Lab Overall Description:**

***Introduction to Transformation -*** Genetic transformation is taking genes from one organism and putting them

in another. A gene is a piece of DNA that gives the instructions for making a protein. This protein gives an organism a

certain trait. A gene is inserted into an organism in order to change the organism’s trait. Genetic transformation is used

in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be

inserted into plants. In medicine, gene therapy treats diseases caused by defective genes by inserting healthy copies of

the defective gene in a sick person’s cells.

You will transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this

gene is a jellyfish. GFP causes certain areas of the jellyfish to glow in the dark. After you transform the bacteria, they

will express their new jellyfish gene and produce the fluorescent protein. It causes them to glow a brilliant green color

under ultraviolet light.

You will learn about the process of moving genes from one organism to another with the aid of a plasmid, a small

circular piece of DNA. Bacteria have one large chromosome and one or more plasmids. Plasmids usually contain genes

for traits that may help the bacteria survive. In nature, bacteria can transfer plasmids back and forth allowing them to

share these beneficial genes. This process allows bacteria to adapt to new environments.

Bio-Rad’s pGLO plasmid has three special genes: one for GFP, a gene for antibiotic resistance, and a gene regulation

system. This system can be used to control when the bacteria produce fluorescent protein. The gene for GFP can be

switched on in transformed cells by adding the sugar arabinose to the cells’ food source. Transformed cells will appear

white on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar

medium. Also, we can test that cells have been transformed with pGLO DNA by growing them on antibiotic plates.

***The Genes -*** Genetic transformation involves the insertion of some new DNA into the E. coli cells. Bacteria

have one large chromosome and one or more plasmids. Plasmids usually contain genes for traits that may help the

bacteria survive. Scientists can use a process called genetic engineering to insert genes coding for new traits into a

plasmid. In this lab, the pGLO plasmid has the GFP gene that codes for the green fluorescent protein and a gene that

codes for a protein that gives the bacteria resistance to an antibiotic. The pGlo plasmid can then be used to transform

bacteria to give them this new trait.

***The Act of Transformation -*** This transformation procedure involves three main steps. These steps are

intended to insert the plasmid DNA into the E. coli cells and provide an environment for the cells to produce their

newly acquired genes.

A. To move the pGLO plasmid DNA through the cell membrane you will:

1.) Use a transformation solution of CaCl2 (calcium chloride) to make cells competent

Competent Cells - Bacterial cells must be in a particular state before they can be transformed. This state is

referred to as *competency*. This state can be achieved naturally in some species of bacteria when levels of nutrients and

oxygen are low. E.coli, the organism on which most current research is performed, must be artificially induced to

make it competent. Competent E. coli cells are very fragile and must be treated carefully.

Transformation Solution: Calcium Chloride CaCl2 - The bacterial cell membrane is permeable to chloride

ions, but is non-permeable to calcium ions. As the chloride ions enter the cell, water molecules accompany the charged

particle. This influx of water causes the cells to swell and is necessary for the uptake of DNA. The exact mechanism of

this uptake is unknown. It is known, however, that the calcium chloride treatment should be followed by heat.

2.) Carry out a procedure referred to as heat shock so bacteria can take in the plasmid

Heat Shock Treatment - When E.coli are subjected to 42°C heat, a set of genes are expressed which aid the

bacteria in surviving at that temperature. This set of genes is called the heat shock genes. The heat shock step is

necessary for the uptake of DNA. At temperatures above 42°C, the bacteria's ability to uptake DNA is lowered, and at

extreme temperatures the bacteria will die.

B. For transformed cells to grow in the presence of ampicillin you must:

3.) Provide nutrients and a short incubation period to begin expressing the newly acquired genes

Incubation - After the heat shock step, intact plasmid DNA molecules replicate in bacterial host cells. To help

the bacterial cells recover from the heat shock, the cells are briefly incubated in LB Nutrient Broth, a solution that

provides nutrients for the bacteria. As the cells recover, plasmid genes are expressed, including those that allow the

replication of plasmids which will end up in new, dividing bacterial cells.



***Genetic Regulation -*** Our bodies contain thousands of different proteins which perform many different jobs.

Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting

us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA

which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human

genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in

your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within

their cells for many reasons, including developmental changes, cellular specialization, and adaptation to the

environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful

overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved

in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the

sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins)

needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when

arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This

regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits

down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered

together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are

called operons. The three genes *(araB, araA* and *araD)* that code for three digestive enzymes involved in the

breakdown of arabinose are clustered together in what is known as the arabinose operon. When arabinose is present in

the environment, bacteria take it up. Once inside, the arabinose interacts directly with arabinose operon and the

interaction causes the transcription of the three digestive enzyme genes. When the three enzymes are produced, they

break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the transcription is shut off.

The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. The

genes which code for break down of arabinose, *araB, A* and *D,* have been replaced by the single gene which codes for

GFP. Therefore, in the presence of arabinose, GFP is produced. Cells fluoresce brilliant green as they produce more

and more GFP. In the absence of arabinose, GFP gene is not transcribed. When GFP is not made, bacteria colonies will

appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.

***Genetic Selection -*** Not all cells will have the plasmid and not all newly produced plasmids will end up in new

bacteria cells. So, it is necessary to select for bacterial cells which contain the plasmid. This is commonly performed

with antibiotic selection. Some E.coli strains cannot grow in the presence of common antibiotics like ampicillin.

Plasmids used for the cloning and manipulation of DNA have been engineered to contain the genes for antibiotic

resistance. Thus, if the bacterial transformation is plated onto media containing ampicillin, only bacteria which have the

plasmid DNA will have the ability to metabolize ampicillin and form colonies. In this way, bacterial cells containing

plasmid DNA are selected.

**Procedure:**

1. At your group’s lab station, check to make sure you have the following materials:

* Test tube labeled, **+pGlo**, containing **250 μl** of **transformation solution** (CaC12)
* Test tube labeled, **-pGlo**, containing **250 μl** of **transformation solution** (CaC12)
* Test tube labeled, **LB,** containing 1ml of **LB Nutrient Broth**

\*The cap of each tube should have your group’s number written on it.

\*The tubes should be in a foam rack on ice.

* One E.coli starter plate (with colonies of E.coli growing on it)
* 4 genetic selection plates (1 LB, 2 LB/Amp, 1 LB/Amp/Ara)
* 1 packet of sterile loops
* 6 sterile pipets in individual packages

*In order to minimize contamination from exposure to the air, ensure all tubes and plates are closed when not in*

*use. When tubes and plates are in use, make sure they are only open for a short time. For plates, only lift the lids*

*enough to insert loops or pipets. Do not completely remove lids and place on the counter-top. This will expose the*

*entire plate to possible contamination.*

2. Use a sterile loop to pick up a single colony of bacteria from your E.coli starter plate.

*In order to avoid contamination, only remove the loop from the package right before you use it and do not touch the*

*loop to any other surfaces besides the starter plate.*

3. Pick up the **+pGLO tube** and place the loop into the transformation solution at the bottom of the tube. Spin the loop

between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no

floating chunks). Place the tube back in the tube rack in the ice.

4. Using a **new sterile loop**, repeat steps 2 and 3 for the **-pGLO tube**.

5. Take your **+pGLO tube** to your instructor to receive the pGlo plasmid. Immerse a **new sterile loop** into the **plasmid**

**DNA stock tube**. Withdraw a loopful. *The loop should have a film on it, as if you were blowing bubbles.* Mix the

loopful into the cell suspension of the **+pGLO tube**. Close the tube and return it to the rack on ice. Close the **-pGLO**

tube. **Do not add plasmid DNA to the -pGLO tube.**

6. Incubate the tubes on ice for 10 minutes. Push the tubes all the way down in the rack so the bottoms of the tubes

make contact with the ice.

7. While the tubes are sitting on ice, label your four agar plates **on the bottom**. Follow the picture below. *Label your*

*tubes as close to the edge as possible so that you can view your results.*

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8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set

at 42 °C, **for exactly 50 seconds**.

9. When the 50 seconds are done, incubate tubes on ice for **2 minutes**.

10. Place the foam tube rack on the bench top. Open a new sterile pipet and add **250 μl of LB nutrient broth** to the

**+pGLO tube**. ***250 μl is the 3rd section up on the pipet.***

*In order to avoid contamination, open the pipet package from the top (near the bulb) and do not touch the pipet tip to*

*any other surfaces.*

11. Repeat step 14 with a new sterile pipet for the **-pGLO tube**.

12. Incubate the tubes for 10 minutes at room temperature.

13. After the 10 minutes incubation, tap the closed tubes with your finger to mix.

14. Using a new sterile pipet for each tube, **pipet 100 μl of the transformation and control** onto the correct plates. ***100***

***μl is the second section up on the pipet.*** *Make sure to pipet the suspensions onto the agar surface of the plates, NOT*

*onto the lids of the plates*

**

**

15. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by skimming

the flat surface of the loop back and forth across the surface. *DO NOT dig the loops into the surface of the agar and*

*create marks on the surface.*

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16. Stack up your plates and tape them together. Put your group number and class period on the bottom of the stack

and place the stack upside down in the 37°C incubator until the next day. Discard all tubes in your waste beaker.

Leave your E.coli starter plate at your station. *Wash your hands thoroughly before leaving class.*



**Predictions:**

Use the chart given below to record your predictions regarding the bacterial growth you will see on each of the plates.

Note: Plates with bacterial growth may show extensive growth of bacteria (i.e. growth wherever the inoculation loop spread the bacteria. This extensive growth is called a bacterial lawn. Plates may also show “dots,” which are colonies of bacteria. The bacteria in these colonies have been successfully “transformed” and are able to live in an environment with ampicillin. Not all bacteria have been successfully transformed, and untransformed bacteria have died, explaining the blank spaces surrounding the colonies on the plate.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Petri Dish** | **Presence of Bacteria** | **Lawn or Colonies?** | **Glow?** | **Picture** | **Explanation** |
| +PGLO  LB / AMP |  |  |  |  |  |
| +PGLO  LB / AMP / Ara |  |  |  |  |  |
| -PGLO  LB/Amp |  |  |  |  |  |
| -PGLO  LB |  |  |  |  |  |

+PGLO = we will be putting bacteria on these plates that have been transformed with the PGLO plasmid

-PGLO = we will be putting bacteria on these plates that have not be transformed with the PGLO plasmid

LB = nutrient broth to feed the bacteria (found on all the plates)

AMP = ampicillin, an antibiotic (should kill off any bacteria that have not been transformed with the PGLO plasmid)

Ara = arabinose, a sugar (should cause any bacteria that have been transformed with the PGLO plasmid to glow green under UV light)

1. On which plate do you expect to find bacteria most like the original E.coli colonies (non-transformed bacteria)?
2. On which plate or plates would you expect to find genetically transformed bacteria?
3. Which plates should be compared to determine if any genetic transformation has occurred?
4. Which plate or plates would be considered control plates in this lab?

**Results:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Petri Dish** | **Presence of Bacteria** | **Lawn or Colonies?** | **Glow?** | **If colonies, how many are present?** |
| +PGLO  LB / AMP |  |  |  |  |
| +PGLO  LB / AMP / Ara |  |  |  |  |
| -PGLO  LB/Amp |  |  |  |  |
| -PGLO  LB |  |  |  |  |

Record your lab results in the table given below.

*\*\*\*Note: Most of the information in this packet is taken from other sources, not written by me (Ms. Ottolini). I have tried to find the original version of these sources and cannot find them in order to cite them. Thank you to whoever created these sources!\*\*\**